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The role of the microenvironment on the fate of adult stem cells

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Adult stem cells (SCs) exist in all tissues that promote tissue growth, regeneration, and healing throughout life. The SC niche in which they reside provides signals that direct them to proliferate, differentiate, or remain dormant; these factors include neighboring cells, the extracellular matrix, soluble molecules, and physical stimuli. In disease and aging states, stable or transitory changes in the microenvironment can directly cause SC activation or inhibition in tissue healing as well as functional regulation. Here, we discuss the microenvironmental regulation of the behavior of SC and focus on plasticity approaches by which various environmental factors can enhance the function of SCs and more effectively direct the fate of SCs.

stem cell, microenvironment, plasticity, fate

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Adult stem cells (SCs) exist in all tissues, where they maintain homeostasis and respond to harmful stimuli. Entrusted with such an important role, these cells are aided by their microenvironment, which provides support and regulatory signals. The SC niche, including cell-cell and cell-extracellular matrix (ECM) relationships as well as diffusible signaling factors, can mediate signal transduction [1,2]. Recent research has shown that adjacent cells provide critical cell-cell interfaces and paracrine signaling and that a number of signaling molecules have been highly conserved from invertebrates to humans [3,4]. Meanwhile, this niche can also reprogram the SCs, as it has been shown that an experimentally vacated ovarian germline stem cell (GSC) niche is a stable structure that is capable of inducing cell division in foreign surrounding somatic SCs that give rise to ovarian follicle cells and is capable of dedifferentiating ectopic follicle progenitor cells in relatively early stages of differentiation [5]. This study also suggested that the SC fate and function could potentially be controlled by microenvironments in *in vitro* systems that artificially recapitulate certain elements of the niche. Because of the important role of SCs in the body and in plasticity, the source of different tissue SCs has been investigated to meet unmet needs in conventional medicine (myocardial infarction, diabetes, cancer, and Parkinson's and Alzheimer's diseases) and regenerative medicine [6–8]. However, SCs usually pass through two different environmental conditions, from isolation to engraftment. It is unknown how microenvironmental effects on SCs constrict their responses to bodily insults and how functional SCs can be domesticated *in vivo*. These questions have led to the hypothesis that, according to various tissue-specific SC niches and different disease microenvironments, mimicking the SC niche will facilitate SC self-renewal and controlled differentiation to drive the fast and efficient differentiation of other SCs so they can join the fight [9].

In this paper, we review the current understanding of the intrinsic environmental characteristics and plastic strategies *in vitro*. By regulating the microenvironment, cell fate can be controlled to enhance SC function to improve the clinical benefits of SC transplantation.

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1 Intrinsic environment characteristics

The SC niche is an anatomical compartment that contains a reservoir of SCs that can maintain normal tissue or replenish injured or aged cell populations in response to mechanisms that regulate whether they should remain quiescent, undergo self renewal, or differentiate [10]. SC niches are composed of four main components: regulatory molecules (O_2, nutrients, and cytokines), other cells (their three-dimensional context, cell-cell contacts, autocrine, and paracrine signals), the extracellular matrix (immobilized and released factors, structure, topology, and stiffness), and physical factors (shear flow, compression, stretch, and electrical signals) [11]. Intrinsic microenvironments orchestrate both systemic and local signals to determine the fate and function of SCs. In this section, we discuss the common elements of SC niches, including cell-cell, cell-ECM, and diffusible signaling factor mediated signal transduction and their regulation of SC fate.

1.1 Cell-cell interactions

Direct interactions between SCs and adjacent cells, either through adherence or gap junctions, are key elements in the native SC microenvironment and are required to modulate SC retention and distribution [12,13]. By observing E-cadherin protein expression in differentiation-defective GSCs, Nagaoka et al. found that unregulated E-cadherin protein expression led to GSC cell proliferation competition, such that differentiated GSCs are forced out of their niche by the downregulation of E-cadherin by neighboring SCs. Such competition with E-cadherin may serve as a quality control mechanism to ensure that the niche is always occupied by functional SCs [14]. Other studies have shown that classical E-cadherins, N-cadherins, are more highly expressed in the brain and are present in neurogenic niches [15−17]. Porlan et al. found that N-cadherin-mediated adhesion to ependymocytes contributes to the quiescence of NSC cells in the SEZ (subependymal zone) niche. At the same time, they also showed that the membrane-type metalloproteinase MT5-MMP, which can cleave N-cadherin in other cell types, regulates the N-cadherin-mediated adhesive properties of neural stem cells (NSCs) under physiological and regenerative conditions and is necessary for the proper activation of NSCs [18]. Furthermore, Ephrin and Notch receptors and their ligands are other classes of integral membrane proteins that are involved in cell contact mediated signaling between SCs and their surroundings [19−21]. For example, Ottone et al. found that direct cell-cell interactions with endothelial cells can enforce quiescence and promote NSCs in the vascular niche of mice through endothelial ephrinB-2 and Jagged-1 mediation. Ephrin-mediated cellular contact between NSCs and their neighboring cells has been proposed to modulate the signaling involved in neurogenesis and NSC self-renewal in the adult brain. Endothelial cells form a critical part of the vasculature niche and involve SC maintenance [21]. Although the direct interactions, through either adherence or gap junctions, between the SCs and adjacent cells are not understood completely, it is believed that such interactions are important to more directly convey information to the SCs or to feedback to the microenvironment cells to participate in repairs or signal regulation.

1.2 Cell-ECM interactions

SCs reside in a dynamic, specialized microenvironment (the SC niche) that provides a three-dimensional extracellular network that surrounds all cells, organs and tissues in the body. This network forms a biophysical filter for protection and nutrition and serves as a medium for facilitating immune responses, angiogenesis and tissue regeneration [22,23]. The niche ECM components, including laminin, fibronectin and collagen, provide a physical framework and instructive signals that regulate SCs. As they serve an adhesion function in the ECM, integrins are involved in the direct binding to a number of ECM components or to other cell surface adhesion molecules and receptors [24,25]. In the epidermis, skin-specific ablation of the β1-integrin gene, attenuated due to an expansion of epidermal SCs following increases in β1-integrin expressing keratinocytes, could be further accelerated in situations of increased keratinocyte proliferation, such as wound healing. In addition, integrins can regulate downstream signaling via focal adhesion kinases (FAKs) and phosphoinositide 3-kinase (PI3K), which are important for SC self-renewal and proliferation [26]. NSCs are embedded in the laminin-rich ECM and express various integrin molecules. Blocking the function of specific integrins in the NSCs using neutralizing monoclonal antibodies enhances precursor proliferation and migration and promotes the depletion of NSCs from the niche [27]. In addition, in a cutaneous epithelial SC microenvironment, Kindlin-1 can trigger integrin to regulate the proliferation and differentiation of cutaneous epithelial SCs by mediating transforming growth factor-β (TGF-β) activation and inhibiting Wnt/β-catenin signaling. This occurs through the integrin-independent regulation of Wnt ligand expression that controls SC quiescence and proliferation [28]. The mechanical properties of the ECM are determined by a network of collagen, fibronectin and fibrin fibrils differing in length and stiffness. Differences in the rigidity of the extracellular environment lead to an intracellular feedback mechanism that regulates the corresponding levels of generated pulling forces and tunes the sensitivity of the focal adhesions to the applied force [29]. Research shows that in longer cultures of up to 14 days, human MSCs (h-MSCs) selectively differentiate based on the culture period before or after stiffening, such that adipogenic differentiation is favored for later stiffening, while osteogenic differentiation is favored for

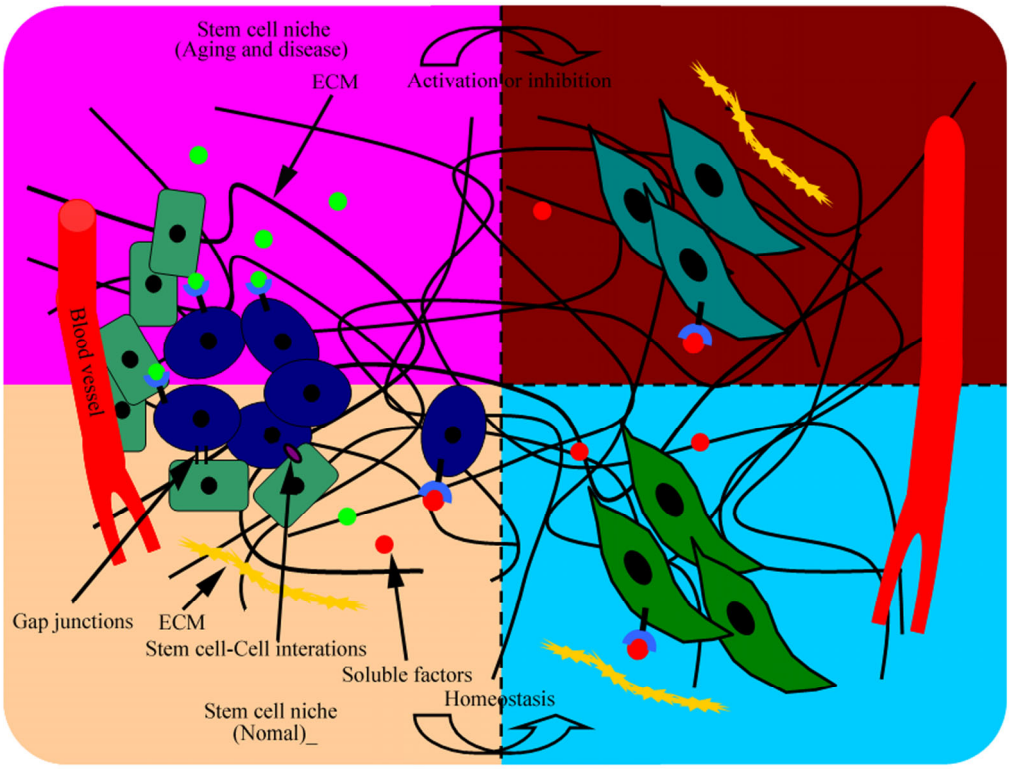

Figure 1 (color online) Schematic representation of the regulatory network of the SC niche that coordinates the balance between stem cell self-renewal and differentiation in normal, aging and disease microenvironments.

earlier stiffening. Changes in the tissue stiffness with time could be highly relevant *in vivo* [30]. Maturation of the mesoderm into adult myocardium results in a considerable increase in the tissue stiffness. When this is mimicked in hydrogels, pre-cardiac cells exhibit increased expression of mature cardiomyocyte markers and form more muscle fibers [31]. This functions to provide signal transduction pathways for SCs by the ECM. By examining SC-ECM interactions at single-cell resolution, it has been possible to map the signaling mechanisms that mediate the different SC responses [32,33]. Some examples of the interplay between integrins and other signaling molecules have been demonstrated in NSCs and in mammary SCs, where β_1-integrins were shown to regulate self renewal and differentiation by controlling the activity of Notch and epidermal growth factor (EGF) receptor [34]. Moreover, β_1-integrins were also found to be essential for regulating the proliferation of intestinal SCs (ISCs) by mediating Hedgehog signaling [35]. By contrast, when human epidermal SCs differentiate on ECM-coated hydrogels of low bulk stiffness, the differentiation signal is mediated by a failure of integrins to cluster in focal adhesions. This results in decreased extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) signaling and increased amphipathic protein-1 (AP1) activity, which is thought to be due to the reduced activity of a phosphatase that acts on JUN N-terminal kinase (JNK) [36]. Thus, the ECM within the SC niche acts to build the niche architecture and control the balance between cell renewal and differentiation by functionally and physically interacting with growth factor and cytokine receptors, thereby generating a network of signaling pathways in the SC niche.

1.3 Cell-soluble protein factor interactions

In the SC niche, soluble protein factors are important elements that regulate SC behavior [37]. Many studies have investigated the role of these cytokines and growth factors, as the immobilization of soluble factors in the ECM plays an important role in mediating their biological effects *in vitro*. Immobilized soluble protein factors can alter their local effective concentration, bioavailability, and stability, thereby modulating their effect on target cells. Previous studies have shown that fibroblast growth factor-2 (FGF2) can activate stress-induced DNA repair to maintain the genomic integrity of keratinocyte SCs [38]. Examples of signaling proteins that are known to undergo such modifications include Sonic hedgehog (Shh) and the wingless-type MMTV integration site family (Wnt), which both have important regulatory functions in neural SC niches [39,40]. Studies show that Wnt3 is strongly expressed in dentate gyrus (DG) hilar cells and in cultured hippocampal astrocytes and that GSK3β/β-catenin-signaling is active in the adult subgranular zone (SGZ) and the dentate granule cell

layer [41]. Notch regulates the maintenance of adult NSCs by promoting cell cycle exit and decreasing the adult neural progenitor pool [42]. TGF-β can inhibit the expansion of NSCs and keep hematopoietic stem cells (HSCs) in their quiescent state, and some studies have shown that TGF-β is critical for the maintenance of the pluripotency of hESCs via Smad2/3 signaling [43]. Thus, soluble factors clearly play an important role in SC growth and differentiation in the stem cell niche. Even if these factors can easily be added or removed from culture systems, their complex mechanisms must still be explored for use in SC applications.

2 Environmental changes: aging and disease

2.1 The aging microenvironment

The regeneration of adult tissues is supported by rare populations of SCs that continuously divide, self-renew and differentiate. This process is tightly regulated by signals emanating from ambient cells to fulfill the dynamic demands of the tissue. One hallmark of aging is slow and aberrant tissue regeneration, which occurs due to the deteriorated function of stem and supporting cells [44]. Telomeres are repetitive nucleotide sequences at the end of each chromosome that allow for DNA replication without a loss of genetic information. Telomere shortening occurs along with organismal aging [45]. In *Drosophila*, it has been found that in aged organisms, 90% of the intestinal gene expression patterns are altered and the ISCs hyper-proliferate and cause aberrant lineage differentiation as well as an accumulation of incorrectly differentiated daughter cells. These changes are largely caused by an accumulation of oxidative stress and are attributed to two signaling pathways: the vascular endothelial growth factor (VEGF) pathway and the Jun N-terminal kinase (JNK) pathway. These studies also partly explain the decreased regenerative capacity of SCs during aging [46–48]. In mammals, Guillot et al. [49] found that MSCs from younger donors have longer telomeres, allowing for more protracted *in vitro* expansion. Aside from a decrease in the overall expansion potential, several groups have also documented that MSCs from older donors have a slower proliferation rate, from the initial cell passage until the culture senesces [50]. Some studies have assessed the differentiation capacity towards the adipo-, osteo- and chondrogenic lineages using bone marrow mesenchymal stem cells (BM-MSCs) of various ages. The frequency of bipotent clones (with osteo- and chondrogenic potential) decreased with age, and a higher relative frequency of bipotent clones was found in younger donors compared to older donors. Conversely, older donors had a relatively higher frequency of tri-potent clones [51]. Several studies have also assessed tissue regeneration in young mice compared to aged mice. Using the distraction osteogenesis model, aged mice were found to have increased circulating serum levels of IL-6 and TNF-α and a 60% reduction in bone formation compared to young mice [52]. SCs reside in a very complex environment that shows age-related changes. All of these factors can easily interfere with the biological properties of SCs, leading SCs to age or senesce by failing to provide the right signals.

2.2 Disease microenvironment

In disease states, activation and inhibition signals in the microenvironment cause continued or transitory changes that directly alter SC function [53]. Hence, SCs show different roles in different diseases, including immune regulation, functional regulation, trophic action, and differentiation. However, along with the continuous disease, the environment of the SCs causes them to gradually lose their function. Humans with type II diabetes have reduced numbers of circulating peripheral blood mononuclear cells that express osteocalcin. They also have elevated levels of serum sclerostin and reduced levels of serum β-catenin. In animal experiments, streptozotocin has been used to break insulin-producing beta cells in the pancreas and thus produces a disease that mimics type I diabetes [54,55]. In a rat model of streptozotocin-induced diabetes, the proliferation of chondroprogenitor cells is similarly reduced at post-fracture days 4 and 7, with decreased cartilage formation and delayed union [56,57]. The potential for endosteal bone formation is also diminished in the streptozotocin-treated diabetic rat model, in which bone growth into titanium-coated femoral implants is reduced compared to the level of osseointegration in control rats [57]. In chronic wound patients, such as patients with diabetes, chronic renal failure, and arterial or venous insufficiency, these effects likely include impaired inflammatory cell migration, reduced growth factor production, and poor tissue remodeling. Rodriguez-Menocal et al. [58] studied fibroblasts derived from normal donors and chronic wound patients that were co-cultured after an in vitro scratch assay. They found that the migration ability was significantly reduced in the fibroblast treatment of BM-MSCs from chronic wound patients compared to MSCs from normal donors. During injury, the support cells are able to activate a repair program, recapitulating aspects of development in the area of damage. These areas become permissive for SC renewal, migration and differentiation. In their research, Ito et al. [59] found that a wound microenvironment can trigger the regeneration of SCs involved in hair follicles by activating the Wnt signaling pathway. The wound microenvironment leads to the activation of certain signaling pathways, driving SC proliferation and differentiation towards wound healing and hair regeneration. The studies also revealed that different diseases inevitably led to changes in the microenvironment, depending on the characteristics of the changing environment and the domestication of functional cells *in vitro*.

3 Management of the microenvironment enhances SC function

SC microenvironments are becoming increasingly appreciated due to their therapeutic effects in regulating SC behavior and homeostasis. In their niche, SCs are maintained or can undergo proliferation and differentiation in response to injury, disease, or aging to replenish lost cells or tissues [6]. In different environments, SCs have different requirements to function properly. Therefore, plastic SCs and progenitor cells have been studied in a variety of environments and have been produced through physical, chemical, genetic, and pharmacological manipulations. In suitable microenvironments, plastic SCs generally have improved cell survival, increased neuronal differentiation, and enhanced paracrine effects, leading to increased trophic support, improved homing to the lesion site, and intensive suppression inflammatory factors and immune responses to promote functional recovery (Figure 2) [60,61]. The microenvironment serves to drive the cells into a state of readiness in different environments.

3.1 Enhanced cell survival

The survival ability of the transplanted cells depends on the treatment effects after the cell is transplanted *in vivo*. The ischemic, oxygen-lacking, inflammatory, cytokine-rich, high glucose, and high adipose microenvironment is harsh, offering a significant challenge to the transplanted donor SCs [61−63]. Massive cell death thus occurs during transplantation and following engraftment, which significantly lowers the effectiveness of the clinical therapy. The simulated hypoxia microenvironment triggers the improved survival of the SCs in vitro and after transplantation [62,64]. Studies show that sub-lethal levels of hypoxia can significantly increase the tolerance of treated cells to apoptotic and other insults *in vitro* as well as in the harsh environments of the ischemic and adjacent regions. BM-MSCs from GFP transgenic mice that were cultured under hypoxic conditions (0.5% oxygen) show significant increases in the expression of hypoxia inducible factor 1 (HIF-1a), angiopoietin-1, erythropoietin (EPO), erythropoietin receptor (EPOR), anti-apoptotic proteins Bcl-2, and Bcl-xL and a decrease in the amount of active caspase-3. The transplantation of MSCs with hypoxic treatment into a region affected by myocardial infarction have a lower level of cell death and apoptosis at 24 h and delayed MSC cell death to 6 W [65]. Another study showed that hypoxia microenvironment induced autophagy could reduce apoptosis of BMSCs in ischemia and hypoxia/serum deprivation (H/SD) environment by increasing the activity of leptin signaling modulate AMPK and mTOR pathway [66]. The lipopolysaccharide (LPS) microenvironment can decrease connexin-43 (CX43) expression in MSCs, enhancing the survival of MSCs under hypoxia and serum deprivation (Hypoxia/SD) conditions. The modulating mechanism may be related to the ERK signaling pathway. Hypoxia and LPS strengthened the power of MSCs against the inflammatory and hypoxia environment, enhancing survival in the target area [67]. The plasticity of the external microenvironment can be effective in enhancing cell survival.

3.2 Enhanced secretion effects

Recent clinical trials have focused on the ability of MSCs to exert their biological function through the paracrine and autocrine actions of secreted cytokines. SCs secrete a variety of factors that support regenerative processes in damaged tissue, induce angiogenesis, protect cells from apoptotic cell death and modulate the immune system [68]. For example, studies have now shown that MSC protein extracts or con-

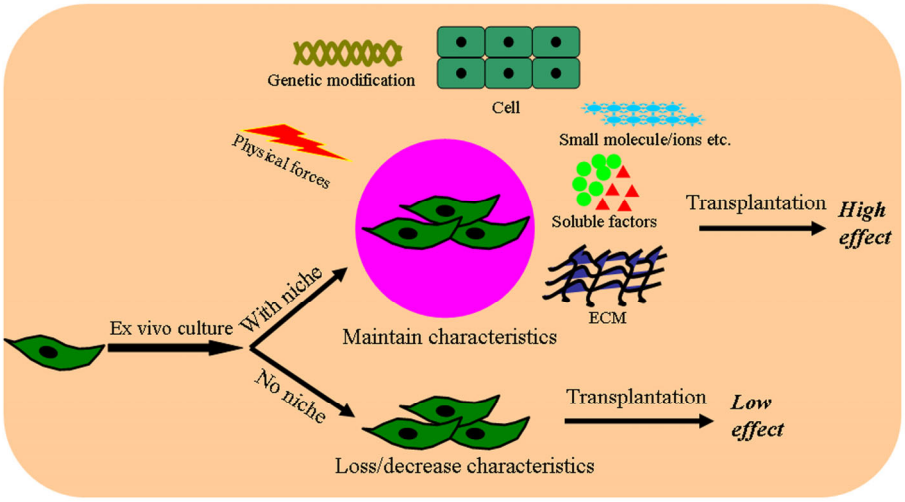

Figure 2 (color online) SC application strategy. SCs are isolated from their native tissue and cultured. The culture conditions were strategically optimized to expand or alter the niches that increase efficiency *in vivo* in transplant recipients.

ditioned medium collected from MSC cultures can produce many of the same beneficial effects as MSCs on the post-infarction heart and in damaged liver tissues [69,70]. MSCs appear to secrete VEGF and basic fibroblast growth factor (bFGF) upon contacting the injured myocardium, which stimulates the formation of new vessels and increases the capillary density to increase/restore blood flow to the infarcted region [71]. However, the secretory activity of MSCs strongly depends on their tissue microenvironmental conditions. After treatment with growth factors, hypoxia or pharmacological agents, plastic MSCs show increased paracrine effects within their microenvironment. Hypoxic exposure increases the expression of stromal cell-derived factor-1 (SDF-1), chemokine receptor-4 (CXCR4), brain-derived neurotrophic factor (BDNF), VEGF, EPO and EPOR in MSCs. These factors have been shown to share the same trophic mechanisms that are essential for the roles of grafted SCs, which are necessary for their survival. Upregulated factors may include angiopoietin-I, VEGF, hepatocyte growth factor (HGF), placental growth factor (PlGF), BDNF, and fibroblast growth factor-2 (FGF-2) [65,72,73]. Treatment using some of these recombinant proteins or the induced endogenous expression of these proteins to enhance the secretion effects can reduce neuronal death and promote angiogenesis and attenuate many pathophysiological changes.

3.3 Enhanced migration and homing

The factors released upon tissue damage or apoptosis mobilize and recruit stem and progenitor cells to the damaged site, where they proliferate and differentiate, eventually replacing the damaged tissues [60]. To respond to migratory signals released in the sites of injury, the SCs must express surface receptors capable of sensing those signals. Thus, increased regenerative and repair potentials were related with the enhanced migration and homing of the SCs to the lesion sites. Various studies attempting to modify the SCs or to enhance their expression of surface receptors to increase SC migration have been performed. Many studies have focused on ways to enhance the functional expression of CXCR4 in MSCs to increase their migration toward chemotactic SDF-1α secreted at injury sites [74]. For example, an environment rich in H_2O_2 is reported to increase the migration of MSCs through the upregulation of CXCR4 and the activation of ERK [75]. Hypoxic conditions can significantly promote the formation of the FAK-Kv2.1 complex, increasing focal adhesion kinase (FAK) phosphorylation and upregulating CXCR4 in MSCs. All of these hypoxic effects reinforce the migration capacity and the homing of transplanted cells to the lesion sites [73]. Maijenburg et al. [76] investigated the process of MSC migration and found that nuclear receptors Nur77 and Nurr1 showed the highest expression in migratory MSCs. The expression of these receptors rapidly increased under stimulation with SDF-1α and platelet-derived growth factor (PDGF). Genetically engineered MSCs overexpressing Nur77 or Nurr1 showed enhanced migration toward SDF-1α.

3.4 Increased differentiation potentials

The differentiation potentials of SCs are increased following migration and homing using tissue transplanted to the lesion sites. Differentiated SC-derived cells, such as osteogenic cells, have been used to treat osteogenic imperfecta patients, as chondrogenic cells in knee cartilage repair, as mesangial cells for the repair of post-glomerular injuries and as myocardiocytes for heart regeneration [77–79]. SC division and differentiation into lineage-specific cells occurs through processes controlled by a combination of the intrinsic fluctuations of protein concentrations and gene state fluctuations occurring through promoter binding. These conditions can be influenced by changes in the microenvironment, including the introduction of chemical agents or growth factors, physical or mechanical stimulation; changes in the cell density, cell-cell attachments and cell-cell interactions; and the direct introduction of regulatory genes into the cells [80–83]. Studies have shown that bone morphogenetic proteins (BMPs) play a significant role by initiating chondrogenesis and differentiation of bone [2]. The ERK1/2 pathway-mediated differentiation of insulin-like growth factor-1 (IGF-1) transfected spinal cord-derived neural SCs into oligodendrocytes. IGF-1 can promote the proliferation and osteogenic differentiation of human dental pulp SCs via the mTOR pathway [84]. Hypoxic preconditioning of the hMSCs can effectively restore osteogenic differentiation, benefitting transplantation therapy for bone regeneration [85]. HIF-1a regulates transcriptional genes involved in the differentiation of bone marrow SCs into cardiomyocytes [86]. Natural and synthetic biomaterials provide three-dimensional differentiation niches to guide the differentiation of SCs towards specific lineages. Bioceramic-collagen scaffolds loaded with human adipose-tissue derived SCs enhanced bone regeneration and reconstruction and also served as appropriate structures in bone regenerative medicine [87]. Furthermore, the combination of biochemical and biophysical stimulatory signals in a three-dimensional setting could potentially enhance the development of mature osteoblasts [88]. A chitosan-gelatin hydrogel containing TGF-β1 was used to promote chondrogenesis, while a polylactic-co-glycolic acid (PLGA) scaffold loading BMP-2 was used to promote osteogenesis [89,90].

3.5 Enhanced immune regulation

Inflammatory and immune responses in tissues may pose secondary and continuous danger to transplanted cells. SCs affect tissue regeneration by modulating inflammation, which has revolutionized SC therapy for the treatment of

inflammatory diseases [63]. For example, the transplantation of SCs for the treatment of myocardial infarction (MI) also suppresses inflammatory responses by significantly decreasing myocardial proinflammatory signaling molecules, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [91]. In fact, different inflammatory states can result in markedly different responses to SC treatment, which indicates the plasticity of MSC immunomodulation. In a non-inflammatory environment, bone marrow derived murine MSCs constitutively express low levels of cyclooxygenase-2 (COX-2), prostaglandin-2 (PGE-2), TGF-β1 and HGF, but not IL-10, programmed death-1 (PD-1), PD-L1 or PD-L2. In a pro-inflammatory environment, interferon-γ (IFN-γ) and IDO expression induced PD-L1 expression, which upregulated and enhanced their immunosuppressive effects after transplantation [92]. We have examined the expression of many pro-inflammatory cytokines/chemokines in BMSCs subjected to hypoxia treatment and observed down-regulated genes, such as CCL4, CXCR3 CXCL10, CC3, CC5 and CC17. In hypoxic preconditioned MSCs, the expression of IL-1β, IL-6, OX-42 and TNF-α is remarkably reduced. After intravenous injection into adult rats 24 h after ischemic stroke, hypoxia-preconditioned BMSCs show a greater ability to suppress microglial activity in the brain compared to normoxia-treated cells [93]. Studies have suggested that the secretion of immunomodulatory factors by MSCs can be enhanced by three-dimensional aggregation or pro-inflammatory cytokine treatment. Ge et al. [94] showed MSC spheroids that were forced to aggregate in agarose can significantly increase the secretion of immunomodulatory paracrine factors, IDO activity and IL-6 secretion.

3.6 Anti-SC aging

SCs are extremely rare in primary tissues. Thus, the *in vitro* expansion of SCs has become an inevitable option. However, after a certain number of cell divisions, SCs enter senescence and stop proliferating, showing an enlarged and flattened cell shape [95]. Previous studies have shown that human MSCs exhibit reduced differentiation potentials *in vitro* and that the replicative senescence of MSC preparations begins with the first passage [51]. Thus, the difficulty in the long-term expansion of MSCs using standard culture systems without the loss of their SC properties suggests that a critical feature of their microenvironment that is necessary for the retention of SC properties is absent in these culture systems. Lai et al. [96] reconstituted a cell-free ECM of native ECM to culture human marrow cells consisting of collagen I and III; fibronectin; small leucine-rich proteoglycans, such as biglycan and decorin; and major components of the basement membrane, such as the large molecular weight proteoglycans perlecan and laminin. The results show that the ECM strongly promoted their proliferation, retained their SC properties with a low level of reactive oxygen species (ROS), and substantially increased their responses to BMP-2. Meanwhile, the bone formation capacity of cells expanded on plastic was dramatically diminished after 6–7 passages [96]. The main components of natural Wharton's jelly extract (WJE) contain ECM proteins, such as collagen and fibronectin, and cytokines, such as IGF-I and bFGF. Using WJE, Hao [97] developed a culture that efficiently suppressed the enhancement of intracellular ROS, p53, and p16INK4a/pRb in MSCs. Their data demonstrated that WJEs can provide the ideal microenvironment for MSC culture expansion *in vitro*, preserving MSC properties by delaying senesce. Another study found that cell contact accelerated the development of replicative senescence during culture. MSCs in contact cultures (with cell passages performed at 100% confluence) reached cellular senescence earlier than in noncontact culture conditions (with subcultures at 60%–70% of confluence), and the doubling time was significantly prolonged. These results show that cell contact induced accelerated senescence in MSCs, accompanied by ROS accumulation due to defective ROS clearance and Ras and p16 (INK4a) upregulation. These changes play an important role in contact-induced senescence in MSCs, independent of telomere shortening and p53 [98]. Cell contact leads to direct cell-cell actions against SC aging *in vitro*, and it remains to be determined whether this is related to cell-cell adherence or gap junctions *in vivo*. The SC microenvironment is dynamic and intricate, and SC behaviors are governed by microenvironment changes *in vivo*. The present study demonstrated that even small changes in the environment will affect the SC fate. Thus, another important remaining question is how to choose suitable microenvironments for the expansion of high quality SCs.

4 Perspectives

Undoubtedly, SC therapy has become an attractive method of regenerative medicine. However, recent clinical trials have focused on the ability of MSCs to exert their biological function through the paracrine and endocrine actions of secreted cytokines. Thus, using the environment to enhance the secretory effect of SCs is increasingly important. Meanwhile, several questions must be answered in the future to improve the current SC applications. (i) How does the use of biomaterials resemble the role of natural ECMs in integrating the building blocks of these complex systems, including the physical properties of the scaffold structure, the spatial and temporal control of bioactive factor delivery, and the construction of a three-dimensional microenvironment to enhance MSC survival and function? (ii) How can the characteristics of disease microenvironments be used to manipulate SC plasticity in vitro and to obtain functional cells with more speed and accuracy? (iii) How can we assist SCs to grow from a young state to a large-scale expansion, or to reverse from an aged to a young state? Continuing to

explore the SC niche will hopefully improve our ability to better characterize SC fate decisions and more effectively develop clinical treatment. Thus, the integration of these different elements will allow us to construct SC microenvironments to better characterize SC fate decisions and more effectively develop robust culture systems.

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